Optimizing everolimus exposure when combined with calcineurin inhibitors in solid organ transplantation

Teun van Gelder [a,*], Lutz Fischer [b], Fuad Shihab [c], Maria Shipkova [d]

[a] Departments of Internal Medicine and Hospital Pharmacy, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands
[b] Department of Hepatobiliary and Transplant Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[c] Division of Nephrology, University of Utah School of Medicine, Salt Lake City, UT, USA
[d] Klinikum Stuttgart, Zentralinstitut für Klinische Chemie und Laboratoriumsmedizin, Stuttgart, Germany

ABSTRACT

The mammalian target of rapamycin (mTOR) inhibitor everolimus is a narrow therapeutic index drug for which optimal exposure levels are essential. The consistent pharmacokinetic profile of everolimus allows trough concentration (C_0) measurement to be an appropriate and reliable index for therapeutic drug monitoring (TDM). Exposure-response analyses of data from early fixed-dose trials demonstrated that rates of biopsy-proven acute rejection (BPAR) are significantly higher if everolimus C_0 declines below 3 ng/mL, an observation confirmed in subsequent concentration-controlled trials. Evidence for the most favorable upper limit is less clear but with reduced-exposure calcineurin inhibitor (CNI) therapy, an upper limit of 8 ng/mL appears to balance efficacy and safety outcomes. The recommended C_0 range is 3–8 ng/mL in kidney, liver and heart transplantation patients, based on LC–MS/MS monitoring in whole blood. Randomized clinical trials based on this target range have demonstrated rates of BPAR comparable to a regimen of mycophenolic acid with standard-exposure CNI. Everolimus exhibits moderate intrapatient pharmacokinetic variability, and it can be challenging to maintain stable concentrations within target range in some individuals. Many factors can influence everolimus exposure for a given dose, including hepatic function, activity of the drug efflux pump P-glycoprotein, the rate of everolimus metabolism, drug–drug interactions (predominantly with CYP3A4 and P-glycoprotein inhibitors, including cyclosporine), intake of fatty food, and patient adherence to the prescribed regimen. Trough concentration levels should be monitored 4–5 days after the first dose and after any change in everolimus dose, with additional monitoring in response to any change in concomitant medication or other clinical circumstances which could alter everolimus exposure. Although LC–MS/MS is the gold standard for everolimus monitoring, various immunoassays are widely used due to their relative simplicity and lower cost, and results can show considerable discrepancies with reference methods due to issues such as interassay variability and cross-reactivity. Method standardization will be important in the future to improve the consistency and reproducibility of results between centers. In conclusion, based on an extensive program of clinical trials, the optimal exposure range for everolimus in combination with reduced-exposure CNI therapy has been established and can be achieved in most transplant recipients through careful, planned TDM.

1. Introduction

Achieving and maintaining optimal immunosuppression after solid organ transplantation represents a complex challenge. The allograft is highly sensitive to underimmunosuppression, which can lead to rejection, while overimmunosuppression can lead to serious and potentially life-threatening complications such as infection and malignancy [1,2]. Transplant recipients frequently have multiple pre-existing or post-transplant comorbidities, such as diabetes [3] and cardiovascular conditions [4], which require numerous medications and further complicate their immunosuppressive drug management.

The mammalian target of rapamycin (mTOR) inhibitor everolimus is approved for the prophylaxis of organ rejection in adult kidney and heart transplant recipients at low to moderate immunological risk [5]. The approval is for everolimus to be administered in combination with low-dose cyclosporine (CsA) and steroids. In practice it is also prescribed with tacrolimus, the more commonly used calcineurin inhibitor (CNI) worldwide. In liver transplantation, everolimus is indicated with low-dose tacrolimus and steroids in recipients at all levels of immunological risk. Similar to CNIs [6–8] and the mTOR inhibitor sirolimus [9,10], everolimus has moderate intra-patient and inter-patient pharmacokinetic variability [11,12], exhibits a clear drug exposure–response relationship [13–15], and has a narrow therapeutic index [16,17]. As a result, the

* Corresponding author at: Dept of Hospital Pharmacy, Room Na-210, Erasmus Medical Center Rotterdam, Wytemaweg 80, 3015 CN, Rotterdam, Netherlands. Tel.: +31 10 7033202; fax: +31 10 7032400.
E-mail address: t.vangelder@erasmusmc.nl (T. van Gelder).

European Medicines Agency (EMA) [18] and the Food and Drug Administration (FDA) [19] recently categorized everolimus as a narrow therapeutic index (NTI) drug in transplantation.

Since the initial studies with everolimus, it has been apparent that maintaining appropriate exposure of this NTI agent is essential. Pharmacodynamic monitoring of post-transplant immunosuppressive regimens, while promising [20], is not yet established and therapeutic drug monitoring (TDM) has underpinned dosing decisions for CNIs for several decades. More recently, it has become clear that TDM is also essential for everolimus therapy in all transplant recipients [21–23]. Because everolimus has linear pharmacokinetics with a strong correlation between trough concentration (C_0) and area under the time-concentration curve (AUC) [12], the pre-dose C_0 represents a simple and reliable index for TDM [21]. A comprehensive review of all aspects of TDM of everolimus was recently published as a Consensus Document by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) [21].

There is now substantial clinical evidence regarding the optimal everolimus exposure level in organ transplant recipients and strategies which may help to achieve this in routine practice. An important caveat is that these data are derived primarily from renal transplantation, with less extensive data from liver or heart transplant recipients despite specific features in these populations (e.g. cholestasis or hypo-dysproteinemia) which could potentially affect the pharmacokinetics of everolimus.

The current evidence base is considered here.

2. Establishing a minimum exposure threshold

The first clinical trials of everolimus in kidney transplantation involved a fixed dose of everolimus with full-dose [24–26] or reduced-dose [24] CsA. Low efficacy failure rates were achieved, and everolimus was generally well tolerated [24, 25]. Pharmacokinetic profiling of everolimus was performed for up to month 6 post-transplant in two randomized double-blind studies [24,26]. A pooled analysis of data from these two clinical trials, published by Kovarik et al. in 2002, showed a significantly higher rate of biopsy-proven acute rejection (BPAR) if everolimus C_0 was below ~3 ng/mL. BPAR occurred in 32% of these patients compared to 14–19% for C_0 in the 3.5–7.7 ng/mL range, and 9% in patients with $C_0 \geq 7.8$ ng/mL [13]. Results from another fixed-dose trial in kidney transplantation demonstrated a clear benefit for reduced-dose CsA versus standard-dose CsA in everolimus-treated patients [25]. Based on these findings, the recommendations are for everolimus dosing to be concentration-controlled using TDM, and for CNI exposure to be reduced in everolimus-treated patients.

In subsequent years, three randomized trials of everolimus with concomitant CsA in de novo kidney transplantation employed an everolimus starting dose of either 1.5 or 3.0 mg/day, with TDM to ensure that everolimus C_0 did not fall below 3 ng/mL [27,28]. Exposure-response analyses based on prospective TDM in these trials confirmed that a C_0 level of 3 ng/mL or higher is associated with a lower incidence of BPAR [15,29]. More specifically, a large analysis of 779 patients by Lorber and colleagues demonstrated that an everolimus C_0 level of ≥3 ng/mL is associated with a reduced risk for early BPAR (i.e. by month 1) and for BPAR by month 6 post-transplant (both p = 0.0001) [15]. By month 6, patients with everolimus $C_0 < 3$ ng/mL had a 3.4-fold higher risk for BPAR compared to those with C_0 in the range 3–8 ng/mL (p < 0.0001) [15]. Moreover, renal allograft survival was higher when everolimus trough level was ≥3 ng/mL (96% versus 80% with $C_0 < 3$ ng/mL).

More recent exposure–response analyses in kidney transplant patients receiving everolimus with either concomitant CsA [14] (Fig. 1) or tacrolimus [30] (Fig. 2) have confirmed the importance of the 3 ng/mL lower cut-off for effective prevention of BPAR.

Only one trial (ASSET), in which 224 de novo kidney transplant patients were randomized to everolimus with either low-exposure levels or very low-exposure levels of tacrolimus, found no significant association
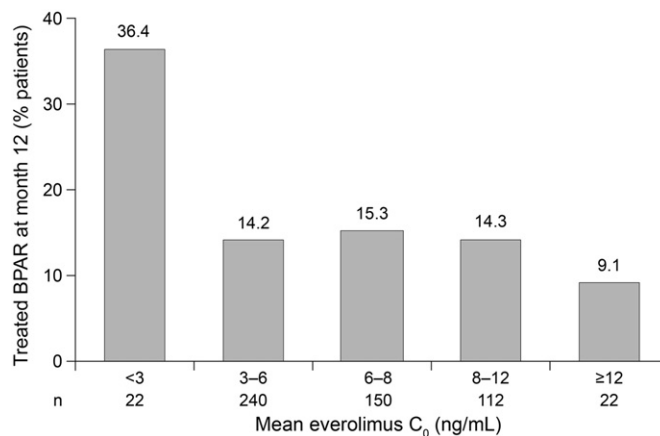

Fig. 1. Incidence of biopsy-proven acute rejection (BPAR) according to everolimus mean trough concentration (C_0) in de novo kidney transplant patients receiving everolimus with reduced-exposure cyclosporine [14].

between everolimus C_0 and the incidence of BPAR. However, exceeding a level of 3 ng/mL still appeared important in the very low-exposure tacrolimus group as the mean everolimus C_0 was only 2.8 ng/mL in patients with BPAR versus 3.5 ng/mL for BPAR-free patients [31].

In de novo liver transplant patients, concentrations below 3 ng/mL were shown to be associated with a high rate of BPAR in a randomized dose-finding trial of 119 patients given everolimus with low-dose CsA [32]. Three-year BPAR rates were 50%, 14% and 12%, respectively, in patients with everolimus $C_0 \leq 3$ ng/mL, 3–6 ng/mL or >6 ng/mL. Lastly, an exposure–response analysis of data from a study in which 634 heart transplant patients were randomized to everolimus at a fixed dose of 1.5 mg/day or 3.0 mg/day, or to a control arm, showed a significant relation between everolimus C_0 and freedom from rejection (p = 0.02) with 3 ng/mL representing a lower threshold for efficacy [33].

3. Evidence for an upper threshold for everolimus exposure

Analyses of the incidence of BPAR according to pharmacokinetic data derived from fixed-dose studies in kidney transplantation [13,29,30] showed a mixed picture regarding an efficacy advantage for high everolimus C_0 levels. An early assessment of data from two studies in which patients were given standard-exposure CsA, found that the incidence of BPAR at month 6 was lowest (9%) with everolimus C_0 in the 8–15 ng/mL range but that BPAR rates were not substantially higher (14–19%) in the 3.5–7.8 ng/mL range [13]. An assessment of 237 patients given concomitant reduced-exposure CsA based on C_2 monitoring (i.e. CsA concentration at two hours post-dose) in the A2306 U.S. everolimus registration trial found no difference in BPAR rates in the groups with everolimus C_0 in the 3–8 ng/mL range versus the >8 ng/mL group (18% in each group), and no significant difference in the risk for graft loss [29]. On the other hand, Lorber and colleagues, in an analysis of 779 de novo CsA-treated kidney transplant patients from two different randomized trials, found the rate of BPAR to be lower with everolimus ≥8 ng/mL versus 3–8 ng/mL (8.4% versus 16.8%) but the number of events in the higher-exposure group (n = 9) was too low to draw any conclusion [15]. With concomitant tacrolimus (maintenance tacrolimus C_0 either 3–6 or 7–10 ng/mL), Chan et al. found no cases of BPAR when everolimus C_0 exceeded 8 ng/mL, but only four patients were in this category [30].

Two randomized clinical trials have prospectively compared outcomes when de novo kidney transplant patients were randomized to one of two different everolimus exposure targets, both with concomitant reduced-exposure CsA [34,35]. A preplanned analysis of data from the large A2309 trial, in which 833 patients were randomized to everolimus targeting 3–8 or 6–12 ng/mL with reduced-exposure CsA (C_0 tapered to 25–50 ng/mL after month 6), or to mycophenolate mofetil (MMF) with
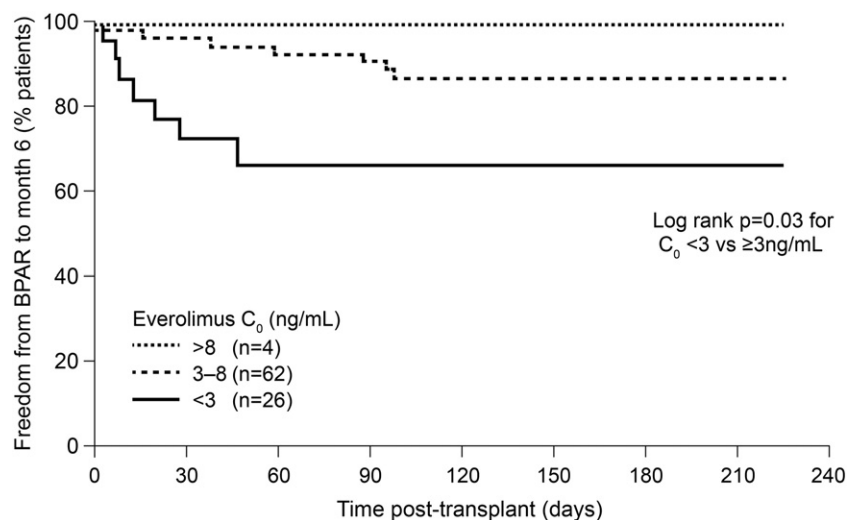
Fig. 2. Incidence of biopsy-proven acute rejection (BPAR) according to everolimus mean trough concentration (C_0) in de novo kidney transplant patients receiving everolimus with reduced-exposure tacrolimus [30].

standard CsA (C_0 tapered to 100–250 ng/mL from month 2) [34], found little difference in BPAR rates when everolimus C_0 was above 8 ng/mL (9.1–14.3%) versus 3–8 ng/mL (range 14.2–15.3%) (Fig. 1) [14]. The second study (EVEREST) randomized newly transplanted kidney patients to everolimus with a target C_0 of 3–8 ng/mL plus low-exposure CsA, or to everolimus targeting 8–12 ng/mL with very low-exposure CsA [35]. BPAR rates were similar between groups (14.7% and 14.1%), but it is difficult to draw conclusions since the CsA exposure in the two groups was also different. For kidney transplantation, the general conclusion is that everolimus concentrations above 8 ng/mL do not provide an additional benefit with regard to efficacy.

In addition, the available data in liver transplant patients receiving concomitant CsA has not indicated a benefit for high everolimus C_0 levels (BPAR 14% versus 12% for C_0 3–6 ng/mL versus >6 ng/mL) [32].

Conversely, everolimus $C_0 > 8$ ng/mL can incur a higher rate of drug-related adverse events, with various reports of higher statin use [30], dyslipidemia [15,29], thrombocytopenia [29], proteinuria [36] and discontinuation due to adverse events [35]. In the A2309 trial, where the two everolimus groups targeted either 3–8 or 6–12 ng/mL, the mean everolimus C_0 after month 3 was approximately 5–6 ng/mL in the lower-exposure group and approximately 8 ng/mL in the higher exposure group, and there was little difference in the adverse event profiles of the two cohorts [34]. A more detailed analysis of the association between everolimus drug exposure and safety in this study, performed by Shihab et al., evaluated the incidence of key adverse events according to mean everolimus exposure categories of <3, 3–6, 6–8, 8–12 and >12 ng/mL [14]. This analysis demonstrated that the lowest rates of wound healing events, peripheral edema and dyslipidemia with everolimus occurred in the range 3–8 ng/mL [14] (Fig. 3). On initial inspection, the 'U-shaped' curve for the incidence of wound healing events, hypercholesterolemia and hypertriglyceridemia (Fig. 3), would argue against a dose-dependent effect. For the lipid parameters, however, closer examination of the data showed that the high incidences in patients with everolimus <3 ng/mL were accounted for by patients who had high CsA exposure, and given the known hyperlipidemic effect of CNI therapy this seems more likely to explain the frequency of lipid abnormalities [14]. For wound healing events, similarly, the frequency of events at low everolimus levels (<3 ng/mL) was again due to high rates in patients with low everolimus/high CsA concentrations [14]. Since wound healing complications are not generally associated with CNIs, one explanation might be that everolimus dose was lowered in response to poor healing, but this is speculative. Peripheral edema at lower everolimus exposure levels did not vary according to CsA concentration [14], and may indicate a relatively dose-insensitive effect of everolimus.

Despite these caveats, evidence for a dose-dependent effect on event rates above 8 ng/mL appears reliable. Of note, where everolimus is used at substantially higher exposure levels for the treatment of solid tumors such as renal cell cancer, meta-analyses have demonstrated substantial increases in the risk for infections overall and for high-grade infections [37,38], as well as for severe anemia [39,40] and other hematological complications including thrombocytopenia [40].

4. Clinical outcomes using the recommended everolimus target range

Based on early exposure–response analyses, and confirmed by later studies, the recommended range for everolimus C_0 in kidney, liver or heart transplant patients is 3–8 ng/mL [5]. This range assumes that patients are receiving concomitant reduced-exposure CNI therapy, as specified in the package insert patient information [5], and applies to everolimus concentrations measured in whole blood by liquid chromatography–tandem mass spectrometry (LC–MS/MS).

Randomized clinical trials in kidney [31,34,35], liver [41] and heart [42] transplant recipients have reported rates of BPAR and other outcomes using everolimus targeting a C_0 in the range 3–8 ng/mL with concomitant low-exposure CsA [34,35] or tacrolimus [30,31,41]. The incidence of BPAR using these regimens has consistently been comparable to a standard regimen of mycophenolic acid (MPA) with standard-exposure CNI (Fig. 4).

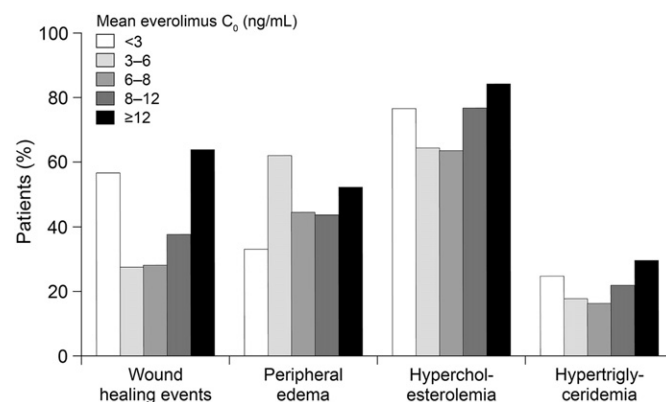**Fig. 3.** Incidence of selected adverse events according to everolimus mean trough concentration (C_0) at month 12 after kidney transplantation in 556 de novo kidney transplant patients receiving everolimus with reduced-exposure cyclosporine [14].
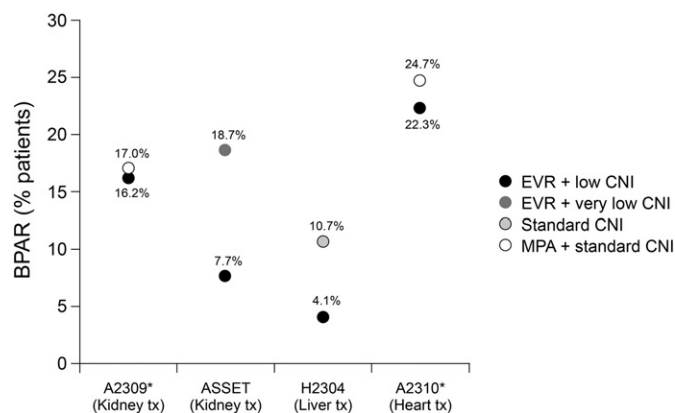
Fig. 4. Incidence of biopsy-proven rejection (BPAR) at month 12 post-transplant in transplant recipients randomized in comparative studies to everolimus (EVR) targeting C_0 3–8 ng/mL with reduced-exposure calcineurin inhibitor (CNI) therapy or to mycophenolic acid (MPA) with standard-exposure CNI in the A2309 [34], ASSET [31], H2304 [41] and A2310 [42] trials. * Treated acute rejection. Tx, transplantation.

Studies investigating the clinical impact of intra-patient variability in everolimus concentrations are lacking. However, a clear association has been demonstrated between higher intra-patient variability with tacrolimus [43,44] or CsA [7] levels and an increased risk for rejection and graft failure. Similarly, a prospective assessment of 51 sirolimus-treated renal transplant patients with chronic allograft nephropathy showed that those with a higher coefficient of variability for sirolimus C_0 (>22.9% versus <22.9%) experienced a significantly greater risk for progressive loss of graft function (p < 0.05) [9]. There is no reason to assume that such an effect does not also apply to everolimus-treated patients.

5. Achieving target everolimus concentrations in clinical practice

Everolimus exhibits moderate pharmacokinetic variability both intra- and inter-patient, and it can be challenging to maintain stable concentrations within target range in some individuals. One study in 706 kidney transplant CsA-treated patients receiving everolimus at an initial dose of 1.5 or 3.0 mg/day observed an inter-patient variability of 55% for everolimus C_0 during the first six months after kidney transplantation, with an AUC variability of 31% [11]. The same study reported the intra-patient variability to be 45% for everolimus C_0 and 27% for AUC [11], while an analysis of 54 maintenance kidney transplant patients found the intra-patient variability for everolimus AUC to be in the range 10–19% [12]. For comparison, values for intra-patient variability in C_0 and AUC, respectively, have been reported to be 36% and 32% for tacrolimus [6], and 40–47% and 37–40% for CsA [7,8], in stable kidney transplant patients. Thus, the observed variability for everolimus C_0 and AUC may be slightly less than for CNI therapies, but nevertheless remains substantial.

Gender, age and body weight do not influence steady-state exposure of everolimus [12]. Evidence for lower everolimus exposure for the same dose in African Americans versus other race groups is mixed [45,46]. CYP3A5 and ABCB1 polymorphisms do not seem to affect everolimus pharmacokinetics to any relevant extent [47]. Other factors, however, exert well-documented effects, particularly hepatic function [48], activity of the drug efflux pump P-glycoprotein, the rate of everolimus metabolism by cytochrome CYP3A4, 3A5 and 2C8 [49], drug–drug interactions (predominantly via CYP3A), intake of fatty food [50], and of course adherence to the prescribed regimen. Perhaps the most important consideration is the concomitant CNI used, whether CsA or tacrolimus. Everolimus, CsA and tacrolimus are metabolized by the cytochrome P450 CYP3A4 and are all substrates for the drug transporter P-glycoprotein [49,51]. However, in vitro studies have shown that although both CsA and tacrolimus block efflux of everolimus from intestinal cells via P-glycoprotein mediated channels to a similar degree, the

effect of CsA on other aspects of everolimus metabolism are more complex than for tacrolimus [52]. CsA, but not tacrolimus, specifically increased permeability — an effect possibly due to inhibition of other transporters or metabolizing enzymes [52]. Additionally, hepatic CYP3A4 may be inhibited to a greater extent by CsA than tacrolimus [19], an effect which would reduce CYP3A4-mediated everolimus clearance. Clinically, everolimus drug exposure is increased by between two- and three-fold in the presence of CsA [53,54]. With tacrolimus, this effect is largely absent [55] such that, compared to patients given concurrent CsA, higher doses of everolimus are required in tacrolimus-treated patients to achieve the same level of everolimus exposure.

While causes of wide variability can be identified in some cases – most notably non-adherence, but potentially also clinical reasons such as changes in concomitant therapies – there is often no explanation for this phenomenon [56]. A TDM simulation of data from two large randomized trials of everolimus with concomitant CsA in kidney transplant patients predicted that a mean of only two everolimus dose adjustments, early after attainment of steady-state levels, would achieve everolimus C_0 > 3 ng/mL in 84% of patients during the first six months post-transplant [15]. However, in practice, and when an upper threshold is targeted in addition to a minimum threshold in order to keep C_0 within a 3–8 ng/mL range, this may not be feasible. For patients with higher intra-patient variability it may be wise to target everolimus trough concentrations at or above the middle of the therapeutic range, since episodes of under-exposure should be avoided as much as possible.

6. Practical aspects of therapeutic drug monitoring for everolimus

Routine TDM monitoring for everolimus, based on chromatographic measurement in whole blood, is essential. Trough concentrations should be monitored 4–5 days after the first dose, and after any change in everolimus dose. The 4–5 days window allows everolimus exposure to reach steady state [12] before making any dose change. As a minimum, everolimus C_0 concentrations should be measured frequently early after transplantation and with longer intervals thereafter as long as everolimus C_0 concentrations remain stable, unless there are triggers such as the ones discussed in the next section.

6.1. Scenarios requiring additional monitoring

Particularly close monitoring is required in certain situations (Table 1). Patients with hepatic dysfunction require particularly watchful dose titration, since almost all elimination of everolimus is via the liver: biliary extraction accounts for 98% of elimination compared to only 2% of urinary excretion [49]. The product license advises that the everolimus dose should be reduced to approximately two-thirds of the normal dose in patients with mild hepatic impairment (Child-Pugh Class A) and by approximately half in patients with moderate or severe hepatic impairment (Child-Pugh B or C) [5], and to be subsequently adjusted as necessary according to TDM. Hepatic dysfunction is also likely to result in

Table 1
Clinical factors influencing everolimus trough concentration (C_0).

Factor	Effect on everolimus C_0
Hepatic dysfunction	⇑⇑⇑
CYP3A4/ABCB1 inhibitors	⇑⇑
CYP3A4/ABCB1 inducers	⇓⇓
CNI therapy	
CsA dose increase	⇑⇑
CsA dose decrease	⇓⇓
Switch from CsA to tacrolimus	⇓⇓⇓
Switch to dispersible tablet form	⇓
Non-adherence	⇓⇓⇓
High-dose steroid therapy	⇓?
Immunoassay cross-reactivity	⇑

slower clearance and a longer elimination half-life, and as a result measured concentrations may not yet reflect steady state.

Any change in concurrent therapy with drugs which are strong inducers or inhibitors of CYP3A4 or ABCB1 necessitates additional monitoring of everolimus exposure [5]. One large analysis in kidney transplant patients has demonstrated the effect of potent CYP3A4 inhibitors: erythromycin or azithromycin therapy reduced everolimus clearance by approximately 20%, with itraconazole reducing clearance by as much as three-quarters, resulting in increases in everolimus blood concentration [46]. A subsequent study demonstrated an even stronger interaction between everolimus and erythromycin, with multiple-dose erythromycin increasing single-dose everolimus blood levels by an average of 4.4-fold [57]. Other CYP3A4 and P-glycoprotein inhibitors such as verapamil [58] and ketoconazole [59] are also associated with well-documented increases in everolimus exposure. The CYP3A4 inducer rifampycin increased everolimus clearance by 172% and reduced AUC by an average of 58% [60]. Accordingly, where treatment with CYP3A and P-glycoprotein inhibitors or inducers is introduced, or their doses are changed, additional monitoring is necessary to adjust the everolimus dose as necessary, guided by TDM results. As discussed above, CsA – which like everolimus is metabolized by CYP3A4 and is a substrate for P-glycoprotein – inhibits everolimus metabolism by approximately 50%, so the everolimus maintenance doses should be 1.5 to 2-fold lower than in tacrolimus-treated patients [53,54,61], and frequent monitoring is required if the CsA dose changes or if the patient is switched to tacrolimus. A possible effect of steroid therapy is less clear-cut, since steroids are substrates, inhibitors and inducers of CYP3A enzymes, and the overall impact may be dose-dependent. Corticosteroids appear to induce the metabolism of tacrolimus [62], and given the similar enzymes involved in the metabolism of everolimus it seems likely that everolimus levels may decrease if high-dose steroids are given. Steroid-tapering steps, similarly, should prompt measurement of everolimus concentrations [61].

6.1.1. Everolimus formulation

Only one formulation of everolimus, Certican® (Zortress® in the USA), is licensed for use in transplantation, but for patients who have difficulty taking standard tablets a dispersible form of Certican® is available. If patients switch between whole tablets and the dispersible formulation, the everolimus concentration may decrease slightly [63] and exposure should be monitored.

Generic versions of NTI drugs such as everolimus are subject to stricter regulatory criteria than other drugs, and any generic preparations of everolimus might need to meet stringent bioequivalence criteria. Nevertheless, if in due course generic formulations were to become available, switching between formulations should be avoided, or if performed must be carefully monitored [64].

6.1.2. Logistic considerations for TDM

Even though pharmacokinetic variability is somewhat lower for everolimus compared to CNIs, the analytical turnaround time for TDM should ideally be the same as for CNI therapy [21]. Prompt analysis minimizes the risk of underexposure in the first days and weeks post-transplant, and of overexposure to everolimus when the clinical situation changes e.g. if the CsA dose is lowered.

Samples can be transported safely in EDTA-anticoagulated tubes to the laboratory unless temperatures and transportation time exceed one week at up to 30 °C or three days at up to 37 °C [21]. If prolonged storage is required, samples can be frozen at −20 °C or below.

6.1.3. Everolimus assays

As reviewed recently [21], different laboratories currently employ different types of assays to measure everolimus concentrations, and the methods have not been standardized. A fully validated LC–MS/MS is the recommended method for measurement of everolimus blood concentration, offering low limits of quantification (≤1 ng/mL) and,

importantly, high analytical specificity for everolimus [21]. Even within the LC–MS/MS method, however, standardization is still required between the different laboratory-developed assays which are available.

Some laboratories use everolimus immunoassays instead of chromatographic techniques. In general, immunoassays require less specialist knowledge than LC–MS/MS methods, and can be run on consolidated clinical chemistry analyzers i.e. instruments upon which a broad spectrum of different laboratory parameters can be measured in parallel. Two commercial immunoassays are available. The first is the Quantitative Microsphere System (QMS® Everolimus assay, Thermo Fisher Scientific/Microgenics), which is approved to monitor everolimus concentrations in samples from kidney and liver transplant recipients, and can be applied to different models of general chemistry analyzers. This has the advantage that laboratories can run the assay on almost any existing instrument, regardless of the manufacturer, but the downside is that this limits the reproducibility of results between the different analytical sites. The second immunoassay is an everolimus assay recently launched by Roche Diagnostics (Elecsys® electrochemiluminescence-immunoassay), which completes their assay menu for immunosuppressive drugs. Although the Elecsys® assay can only be used by laboratories that already have the relevant Roche equipment, since it is not compatible with other instruments, it provides a more advanced form of assay standardization. Both immunoassays show cross-reactivity to everolimus metabolites, but the extent of cross-reactivity to single metabolites varies between assays [65,66]. Moreover, the two assays rely on different calibration strategies. Calibration of the Roche assay is gravimetric, similar to LC–MS/MS methods, which makes control of accuracy easier for the laboratories, but due to the cross-reactivity with metabolites it overestimates everolimus concentrations. In contrast, the QMS® assay factors up the concentrations of calibrators artificially to compensate for cross-reactivity with metabolites and to make readings more similar to LC–MS/MS methods. Although this approach is more convenient at first glance, it bears the risk of misinterpretation. It is only valid for population averages and can produce wide deviations from reference LC–MS/MS concentrations for individual patients, as recognized by the manufacturer [65].

In particular, everolimus quantification in samples from patients with an aberrant drug metabolism or an unusual pattern of metabolites may be affected [21]. A recent analysis in kidney, liver and heart transplant patients confirmed that everolimus concentrations are not consistent between the three methods: the Elecsys® assay overestimated the concentration compared to LS-MS/MS while QMS® showed a small but significant negative deviation [67]. As a consequence of these two different calibration strategies, higher therapeutic ranges should be applied to Roche immunoassay results compared to those from QMS® or LC–MS/MS.

Finally, it should be borne in mind that both immunoassays are affected by cross-reactivity to structurally-related substances e.g. to sirolimus. Therefore, no accurate TDM of everolimus is possible with either immunoassay during the first week after switching from sirolimus therapy to everolimus when both drugs are present in the blood sample.

As a consequence of these analytical issues, laboratory measurements of everolimus concentrations using different techniques lack consistency and their results cannot be considered interchangeable. This is true even with ongoing participation in an external proficiency testing program, although it is still strongly recommended regardless of the assay type that laboratories use to continuously cross-validate results with a reference method, check the analytical quality, and control for calibration bias [21].

In the future, method standardization may help to improve the consistency of results between laboratories.

7. Conclusion

Since it was first developed in the 1990s, everolimus-based immunosuppression in transplant recipients has been researched in a large number of well-designed trials. The efficacy benefit for maintaining

everolimus C_0 level at 3 ng/mL or higher in patients receiving concomitant reduced-exposure CNI therapy is well-documented. The upper limit of 8 ng/mL in the product license avoids an increased risk for dose-dependent mTOR inhibitor-related adverse effects without loss of efficacy compared to lower exposure levels. The bulk of data are based on studies in kidney transplantation, but although data from liver and heart transplant patients are more limited there is no indication of a marked difference in the optimal target range between organ types.

A planned schedule of regular TDM assessments, with additional measurements in response to various established influences on everolimus exposure is a mandatory part of the management protocol. Stability of exposure can be helped by ensuring that patients take everolimus as indicated, i.e. consistently either with or without food and at the same time as the CNI therapy. Where everolimus C_0 varies widely despite a steady dose, without obvious explanations for excessive variation, adherence to the prescribed regimen should be investigated. Decision-making regarding the method of everolimus TDM will frequently depend on the available expertise and financial constraints, but regardless of the type of assay it is a priority to establish a rapid turnaround of sample results and to maintain regular validation of measurements versus a reference laboratory.

A long program of research into everolimus has refined our understanding of how to optimize its use. Although practical challenges remain in achieving adequate therapeutic exposure in every patient, the imperative is clear and vigilant monitoring via an appropriate assay technique is a priority.

References

[1] Cantarovich D, Vistoli F, Soulillou JP. Immunosuppression minimization in kidney transplantation. Front Biosci 2008;13:1413–32.

[2] Rama I, Grinyó JM. Malignancy after renal transplantation: the role of immunosuppression. Nat Rev Nephrol 2010;6:511–9.

[3] Vincenti F, Friman S, Scheuermann E, et al, DIRECT (Diabetes Incidence after Renal Transplantation: Neoral C Monitoring Versus Tacrolimus) Investigators. Results of an international, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. Am J Transplant 2007;7:1506–14.

[4] Stoumpos S, Jardine AG, Mark PB. Cardiovascular morbidity and mortality after kidney transplantation. Transpl Int 2015;28:10–21.

[5] Certican® (everolimus) Prescribing Information. Novartis Pharma AG, Basel, Switzerland.

[6] Higgins RM, Hart P, Lam FT, Kashi H. Conversion from tacrolimus to cyclosporin in stable renal transplant patients: safety, metabolic changes, and pharmacokinetic comparison. Transplantation 2000;70:199–202.

[7] Kahan BD, Welsh M, Urbauer DL, et al. Low intraindividual variability of cyclosporin A exposure reduces chronic rejection incidence and health care costs. J Am Soc Nephrol 2000;11:1122–31.

[8] Kahan BD, Welsh M, Schoenberg L, et al. Variable oral absorption of cyclosporine. A biopharmaceutical risk factor for chronic renal allograft rejection. Transplantation 1996;62:599–606.

[9] Wu MJ, Shu KH, Lian JD, Yang CR, Cheng CH, Chen CH. Impact of variability of sirolimus trough level on chronic allograft nephropathy. Transplant Proc 2008;40:2202–5.

[10] Jiao Z, Shi XJ, Li ZD, Zhong MK. Population pharmacokinetics of sirolimus in de novo Chinese adult renal transplant patients. Br J Clin Pharmacol 2009;68:47–60.

[11] Kovarik JM, Kaplan B, Silva HT, et al. Pharmacokinetics of everolimus-cyclosporine immunosuppressive regimen over the first 6 months after kidney transplantation. Am J Transplant 2003;3:606–13.

[12] Budde K, Fritsche L, Waiser J, Glander P, Slowinski T, Neumayer HH. RADW 102 Renal Transplant Study Group. Pharmacokinetics of the immunosuppressant everolimus in maintenance renal transplant patients. Eur J Med Res 2005;10:169–74.

[13] Kovarik JM, Kaplan B, Tedesco Silva H, et al. Exposure-response relationships for everolimus in de novo kidney transplantation: defining a therapeutic range. Transplantation 2002;73:920–5.

[14] Shihab FS, Cibrik D, Chan L, et al. Association of clinical events with everolimus exposure in kidney transplant patients receiving reduced cyclosporine. Clin Transplant 2013;27:217–26.

[15] Lorber MI, Ponticelli C, Whelchel J, et al. Therapeutic drug monitoring for everolimus in kidney transplantation using 12-month exposure, efficacy, and safety data. Clin Transplant 2005;19:145–52.

[16] Budde K, Neumayer HH, Lehne G, Winkler M, Hauser IA, Lison A. Et al; RADW 102 Renal Transplant Study Group. Tolerability and steady-state pharmacokinetics of everolimus in maintenance renal transplant patients. Nephrol Dial Transplant 2004;19:2606–14.

[17] Kovarik JM, Kahan BD, Kaplan B, et al. Longitudinal assessment of everolimus in de novo renal transplant recipients over the first post-transplant year: pharmacokinetics, exposure-response relationships, and influence on cyclosporine. Clin Pharmacol Ther 2001;69:48–56.

[18] European medicines agency EMG/CHMP/154772/2016 Committee for Medicinal Products for human use (CHMP) [1 April 2016] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/05/WC500205726.pdf

[19] Food and Drug Administration. Draft guidance on everolimus. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM506884.pdf; 2016.

[20] Barraclough KA, Staatz CE, Isbel NM, McTaggart SJ. Review: Pharmacodynamic monitoring of immunosuppression in kidney transplantation. Nephrology (Carlton) 2010;15:522–32.

[21] Shipkova M, Hesselink DA, Hold DW, et al. Therapeutic drug monitoring of everolimus: a consensus report. Ther Drug Monit 2016;38:143–69.

[22] Moes DJ, Guchelaar HJ, de Fijter JW. Sirolimus and everolimus in kidney transplantation. Drug Discov Today 2015;20:1243–9.

[23] Mabasa VH, Ensom MH. The role of therapeutic monitoring of everolimus in solid organ transplantation. Ther Drug Monit 2005;27:666–76.

[24] Kahan BD, Kaplan B, Lorber MI, Winkler M, Cambon N, Boger RS. RAD in de novo renal transplantation: comparison of three doses on the incidence and severity of acute rejection. Transplantation 2001;71:1400–6.

[25] Nashan B, Curtis J, Ponticelli C, Mourad G, Jaffe J. Haas T; 156 study group. Everolimus and reduced-exposure cyclosporine in de novo renal-transplant recipients: a three-year phase II, randomized, multicenter, open-label study. Transplantation 2004;78:1332–40.

[26] Vitko S, Margreiter R, Weimar W, et al. RAD B201 study group. Everolimus (Certican) 12-month safety and efficacy versus mycophenolate mofetil in de novo renal transplant recipients. Transplantation 2004;78:1532–40.

[27] Vitko S, Tedesco H, Eris J, et al. Everolimus with optimized cyclosporine dosing in renal transplant recipients: 6-month safety and efficacy results of two randomized studies. Am J Transplant 2004;4:626–35.

[28] Lorber MI, Mulgaonkar S, Butt KM, et al. Everolimus versus mycophenolate mofetil in the prevention of rejection in de novo renal transplant recipients: a 3-year randomized, multicenter, phase III study. Transplantation 2005;80:244–52.

[29] Kovarik JM, Tedesco H, Pascual J, et al. Everolimus therapeutic concentration range defined from a prospective trial with reduced-exposure cyclosporine in de novo kidney transplantation. Ther Drug Monit 2004;26:499–505.

[30] Chan L, Hartmann E, Cibrik D, Cooper M, Shaw LM. Optimal everolimus concentration is associated with risk reduction for acute rejection in de novo renal transplant recipients. Transplantation 2010;90:31–7.

[31] Langer RM, Hené R, Vitko S, et al. Everolimus plus early tacrolimus minimization: a phase III, randomized, open-label, multicentre trial in renal transplantation. Transplant Int 2012;25:592–602.

[32] Levy G, Schmidli H, Punch J, et al. Safety, tolerability, and efficacy of everolimus in de novo liver transplant recipients: 12- and 36-month results. Liver Transpl 2006;12:1640–8.

[33] Kovarik JM, Eisen H, Dorent R, et al. Everolimus in de novo cardiac transplantation: pharmacokinetics, therapeutic range, and influence on cyclosporine exposure. J Heart Lung Transplant 2003;22:1117–25.

[34] Tedesco-Silva Jr H, Cibrik D, Johnston T, et al. Everolimus plus reduced-exposure CsA versus mycophenolic acid plus standard-exposure CsA in renal-transplant recipients. Am J Transplant 2010;10:1401–13.

[35] Salvadori M, Scolari MP, Bertoni E, et al. Everolimus with very low-exposure cyclosporine A in de novo kidney transplantation: a multicenter, randomized, controlled trial. Transplantation 2009;88:1194–202.

[36] Bertoni E, Larti A, Rosso G, Zanazzi M, Di Maria L, Salvadori M. Good outcomes with cyclosporine very low exposure with everolimus high exposure in renal transplant patients. J Nephrol 2011;24:613–8.

[37] Je Y, Sonpavde G, Galsky MD, et al. Incidence and risk of infections in renal cell cancer (RCC) and non-RCC patients treated with everolimus and temsirolimus: a meta-analysis of randomized control trials. J Clin Oncol 2013;31(Suppl. 6):353.

[38] Garcia CA, Wu S. Attributable risk of infection to mTOR inhibitors everolimus and temsirolimus in the treatment of cancer. Cancer Invest 2016;34:521–30.

[39] Shameem R, Hamid MS, Wu S. Risk of anemia attributable to everolimus in patients with cancer: a meta-analysis of randomized controlled trials. Anticancer Res 2015;35:2333–40.

[40] Xu J, Tian D. Hematologic toxicities associated with mTOR temsirolimus and everolimus in cancer patients: a systematic review and meta-analysis. Curr Med Res Opin 2014;30:67–74.

[41] De Simone P, Nevens F, De Carlis L, et al. H2304 study group. Everolimus with reduced tacrolimus improved renal function in de novo liver transplant recipients: a randomized controlled trial. Am J Transplant 2012;12:3008–20.

[42] Eisen HJ, Kobashigawa J, Starling RC, et al. Everolimus versus mycophenolate mofetil in heart transplantation: a randomized, multicenter trial. Am J Transplant 2013;13: 1203–16.

[43] Borra LC, Roodnat JI, Kal JA, Mathot RA, Weimar W, van Gelder T. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation. Nephrol Dial Transplant 2010;25:2757–63.

[44] Sapir-Pichhadze R, Wang Y, Famure O, Li Y, Kim SJ. Time-dependent variability in tacrolimus trough blood levels is a risk factor for late kidney transplant failure. Kidney Int 2014;85:1404–11.

[45] Taber DJ, Belk L, Meadows H, et al. Racial comparisons of everolimus pharmacokinetics and pharmacodynamics in adult kidney transplant recipients. Ther Drug Monit 2013; 35:753–9.

[46] Kovarik JM, Hsu CH, McMahon L, Berthier S, Rordorf C. Population pharmacokinetics of everolimus in de novo renal transplant patients: impact of ethnicity and comedications. Clin Pharmacol Ther 2001;70:247–54.

[47] Moes DJ, Press RR, den Hartigh J, van der Straaten T, de Fijter JW, Guchelaar HJ. Population pharmacokinetics and pharmacogenetics of everolimus in renal transplant patients. Clin Pharmacokinet 2012;51:467–80.

[48] Peveling-Oberhag J, Zeuzem S, Yong WP, et al. Effects of hepatic impairment on the pharmacokinetics of everolimus: a single-dose, open-label, parallel-group study. Clin Ther 2013;35:215–25.

[49] Kirchner GI, Meier-Wiedenbach I, Manns MP. Clinical pharmacokinetics of everolimus. Clin Pharmacokinet 2004;43:83–95.

[50] Kovarik JM, Hartmann S, Figueiredo J, et al. Effect of food on everolimus absorption: quantification in healthy subjects and a confirmatory screening in patients with renal transplants. Pharmacotherapy 2002;22:154–9.

[51] Masuda S, Inui K. An up-date review on individualized dosage adjustment of calcineurin inhibitors in organ transplant patients. Pharmacol Ther 2006;112:184–98.

[52] Lamoureux F, Picard N, Boussera B, Sauvage FL, Marquet P. Sirolimus and everolimus intestinal absorption and interaction with calcineurin inhibitors: a differential effect between cyclosporine and tacrolimus. Fundam Clin Pharmacol 2012;26:463–72.

[53] Kovarik JM, Curtis JJ, Hricik DE, Pescovitz MD, Scantlebury V, Vasquez A. Differential pharmacokinetic interaction of tacrolimus and cyclosporine on everolimus. Transplant Proc 2006;38:3456–8.

[54] Brandhorst G, Tenderich G, Zittermann A, et al. Everolimus exposure in cardiac transplant recipients is influenced by concomitant calcineurin inhibitor. Ther Drug Monit 2008;30:113–6.

[55] Rostaing L, Christiaans MH, Kovarik JM, Pascual J. The pharmacokinetics of everolimus in de novo kidney transplant patients receiving tacrolimus: an analysis from the randomized ASSET study. Ann Transplant 2014;19:337–45.

[56] van Gelder T. Within-patient variability in immunosuppressive drug exposure as a predictor for poor outcome after transplantation. Kidney Int 2014;85:1267–8.

[57] Kovarik JM, Beyer D, Bizot MN, Jiang Q, Shenouda M, Schmouder RL. Effect of multiple-dose erythromycin on everolimus pharmacokinetics. Eur J Clin Pharmacol 2005;61:35–8.

[58] Kovarik JM, Beyer D, Bizot MN, Jiang Q, Allison MJ, Schmouder RL. Pharmacokinetic interaction between verapamil and everolimus in healthy subjects. Br J Clin Pharmacol 2005;60:434–7.

[59] Kovarik JM, Beyer D, Bizot MN, Jiang Q, Shenouda M, Schmouder RL. Blood concentrations of everolimus are markedly increased by ketoconazole. J Clin Pharmacol 2005;45:514–8.

[60] Kovarik JM, Hartmann S, Figueiredo J, Rouilly M, Port A, Rordorf C. Effect of rifampin on apparent clearance of everolimus. Ann Pharmacother 2002;36:981–5.

[61] Christians U, Jacobsen W, Benet LZ, Lampen A. Mechanisms of clinically relevant drug interactions associated with tacrolimus. Clin Pharmacokinet 2002;41:813–51.

[62] Anglicheau D, Flamant M, Schlageter MS, et al. Pharmacokinetic interaction between corticosteroids and tacrolimus and renal transplantation. Nephrol Dial Transplant 2003;18:2409–14.

[63] Kovarik JM, Noe A, Berthier S, et al. Clinical development of an everolimus pediatric formulation: relative bioavailability, food effect, and steady-state pharmocokinetics. J Clin Pharmacol 2003;43:141–7.

[64] van Gelder T. What is the future of generics in transplantation? Transplantation 2015;99:2269–73.

[65] Thermo Fisher Scientific Inc.. QMS® everolimus immunoassay package insert. Fremont, CA, USA: Thermo Fisher Scientific, Inc.; 2015.

[66] Roche Diagnostics GmbH. Cobas® everolimus immunoassay package insert (4; revision 1)[Mannheim, Germany] ; 2016.

[67] Shipkova M, Rapp S, Rigo-Bonnin R, Wieland E, Peter A. Therapeutic drug monitoring of everolimus: comparability of concentrations determined by two immunoassays and a LC-MS/MS method. Ther Drug Monitor 2017 [Epublication ahead of print].